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Transplantation for AML in Children

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INTRODUCTION

Jean Sanders and colleagues at the Fred Hutchinson Cancer Research Center reported in 1985 that sibling donor transplant in first remission is the optimal treatment for childhood acuty myelogenous leukemia (AML), with disease-free survival of 64%, and excellent quality of life [1]. This seminal observation remains largely, although not entirely, true 26 years later. Current movement in the application of transplant in AML is in 2 contrasting directions. Physicians are now withholding transplant from children with very good-risk disease, and trying to use transplant earlier in children with the highest risk disease.

Over the last quarter century important progress has been made in the use of chemotherapy for AML, in series of randomized clinical trials conducted by cooperative groups in the United States, Europe, and elsewhere. The key strategies that have improved survival with chemotherapy are successful dose intensification, made possible with improved aggressive supportive care and improved risk group assignment. Improvements in chemotherapy and in risk assignment now allow us to identify children with favorable biology (those with t[8:21], or inversion 16) for whom allogeneic transplant in first complete remission (CR1) is unnecessary [2,3].

A key achievement in the last 25 years has been the easy availability of well-matched unrelated donors from well-integrated registries, and of banks of frozen cord blood [4,5]. Many studies now report that results comparable to those seen with sibling donors can be achieved with unrelated donor stem cells, and this extends availability of transplant to many more children. These advances are remarkable, and yet there remains significant room for improvement in transplantation for childhood AML, as morbidity and mortality, together with late effects, remain considerable. Moreover, it remains unclear if children with the highest risk disease benefit from transplantation, and efforts are currently being made to answer these questions. This presentation will focus on potential strategies to further improve transplant outcomes in cases with high-risk AML.

IDENTIFICATION OF HIGHEST RISK PATIENTS

Our approach that should be considered for stem cell transplantation in first CR has evolved over the last 2 decades. Woods et al. [6,7] demonstrated that patients who received an allogeneic stem cell transplantation from a matched family donor in first CR had an improved outcome compared with conventional chemotherapy. As a result of this study, stem cell transplantation (SCT) from an HLA matched family donor in first CR became the standard of care for treatment of childhood AML. As patients with −7 and −5/del5q were found to have an extremely poor outcome with conventional chemotherapy, more recent trials allocated this small cohort of high-risk patients to SCT from the most suitable donors. Further, patients with favorable risk cytogenetics (Core Binding Factor AML), were shown to have similar outcomes regardless of whether they are treated with chemotherapy or SCT. Thus, those with Core Binding Factor AML would not receive an allogeneic SCT in first CR even if a matched donor was available. In this 3-tier risk-based therapy allocation, 5% to 10% of the patients would be considered high risk, 20% would be in the favorable risk category, and the majority of patients would remain in the standard risk category and would receive SCT in first CR if a matched family donor was available. More recently, several AML-associated mutations were correlated with clinical outcome, thus expanding the risk cohorts for therapy allocation. Mutations in the NPM and CEBPA genes are shown to be associated with improved clinical outcome [8,9] and those with FLT3/ITD associated with poor outcome with conventional chemotherapy [10]. Addition of these mutations increased high- and low-risk cohorts by 10% each, and the standard-risk cohort remained at nearly 65%. Multidimensional flow cytometry (MDF) has been used to define relapse risk in those without known cytogenetic or molecular markers. COG

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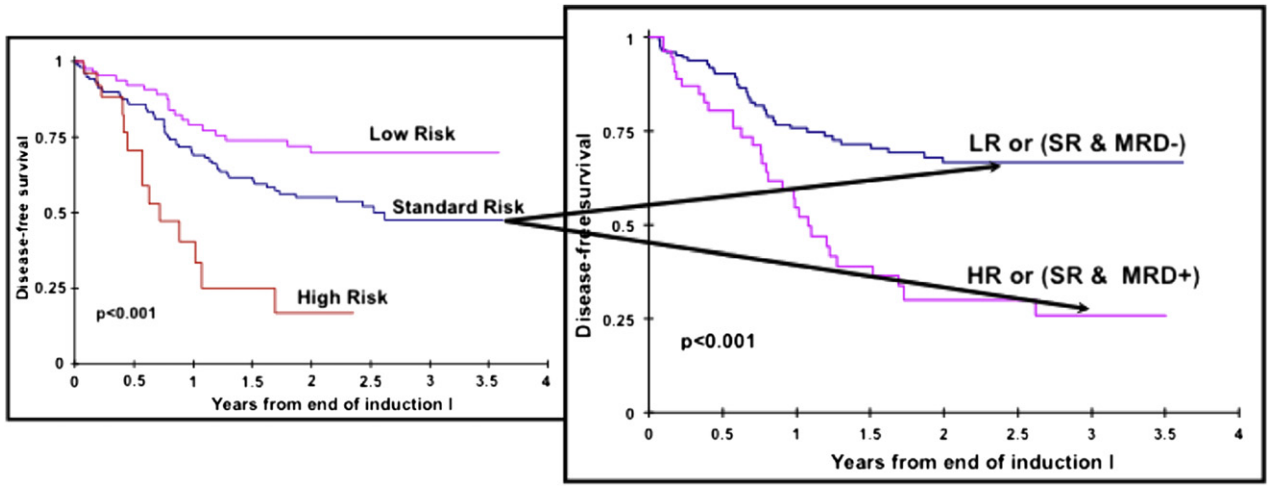

Figure 1. Two-tier risk allocation schema. Presence of residual disease at the end of induction is associated with relapse and poor outcome in standard risk, but not in the high- or low-risk patients. Addition of MDF data in SR cohort to the previously known cytogenetics/molecular prognostic data allows generation of 2-tier schema that allows risk appropriate allocation of SCT in first CR.

AML protocol AAML03P1 collected comprehensive cytogenetics characteristics, mutation profile (*FLT3/*ITD, *CEBPA*, and *NPM* mutation status), and MDF data, and demonstrated that in patients without known molecular prognostic markers (standard-risk [SR] cohort), MDF was able to identify presence of minimal residual disease (MRD) in nearly 30% of patients. In this SR cohort, those with MRD after induction had a significantly worse survival than those who did not have evidence of MRD by MDF (26% versus 67%). This study also demonstrated that presence of MRD in those with high-risk or low-risk disease was not associated with outcome [11]. As a result, a novel 2-tier risk-based allocation system has been devised and implemented into the COG AML trials, where those with either high-risk cytogenetic and molecular features or those with otherwise SR disease with MRD are allocated to the high-risk arm of the study and receive SCT from the most suitable donor in first CR. The remaining patients, those with favorable cytogenetics/mutations or the MRD-negative SR cohort are considered favorable risk and would not receive SCT in first CR regardless of availability of matched family donors (Figure 1).

Cytogenetics, molecular genotyping, and postinduction MDF analysis provide a robust means of stratifying all pediatric patients with AML into 2 risk groups with significantly different outcomes. This novel risk-based approach enables assignment of SCT to those at highest risk of relapse who may most benefit from this aggressive management.

APPROACHES TO IMPROVING OUTCOMES: SELECTION OF OPTIMAL DONORS

Seventeen percent of children with AML and primary induction failure were long-term survivors in a report describing unrelated donor transplants for AML [12]. These data suggest that this small subset of children who were clearly refractory to chemotherapy achieved disease control from the presence of allogeneic cells. This raises the tantalizing prospect that if we knew how to select the optimal donor we would be able to control disease easily in all children with refractory disease. The use of killer cell immunoglobulin-like receptor (KIR) mismatch to select donors that are likely to control disease without causing fulminant graft-versus-host disease (GVHD) has been proposed and supported by mouse models and some, but not all, clinical data [13-19]. The validity of this strategy, and the optimal way to examine KIR genotype and phenotype to make the donor selection, remain controversial [20-22]. A prospective COG study is seeking to address this issue prospectively, asking whether it is feasible to obtain KIR typing in a timely manner for unrelated donor and cord blood transplants, and whether outcomes are improved.

About half of all transplants for pediatric leukemia now use cord blood as a stem cell source. Preliminary studies from the University of Minnesota suggest that relapse is reduced in recipients of double cord blood transplants, compared with single-unit transplantations, and this question is being addressed by the bone marrow transplant clinical trials network in a prospective randomized trial, close to completion of accrual [23].

APPROACHES TO IMPROVING OUTCOMES: IS THERE BENEFIT TO IRRADIATION OR INCREASING DOSE INTENSITY?

Busulfan (Bu) and cyclophosphamide (Cy) as preparative therapy has become a standard approach to transplant for children with AML, and the availability of intravenous busulfan has facilitated this treatment.

The role of radiation as perhaps a superior modality for disease control remains an issue, as the focus of transplantation is increasingly on control of high-risk disease. Four largely adult randomized trials comparing Bu/Cy with total-body irridiation (TBI) have shown no statistically significant difference in survival [24]. Similarly, a European Bone and Marrow Transplant (EBMT) comparison of outcomes reported to their registry also showed similar outcomes with Bu/Cy or Cy/TBI [25]. Similar pediatric registry or prospective studies are not available so some uncertainty remains, but the increased adverse effects associated with use of TBI in young children, and the absence of compelling data suggesting improved disease control in the adult studies limit enthusiasm. A number of studies, most conducted some years ago, have sought to add additional chemotherapy to TBI, increased the dose of TBI, or replaced cyclophosphamide with an alternative alkylating agent [26,27]. None of these studies showed improved survival, with the majority showing modest reduction in relapse offset by increased treatment-related mortality (TRM), suggesting little yield from intensification of the preparative regimen.

APPROACHES TO IMPROVING OUTCOMES—DOES REDUCING THE INTENSITY OF THE PREPARATIVE REGIMEN MAKE SENSE FOR PEDIATRIC AML?

The use of reduced-intensity conditioning (RIC) regimens for older adults with AML has been an important advance, and has extended transplantation to persons typically thought ineligible. A number of authors have sought to compare these 2 strategies, generally in a retrospective nonrandomized manner, and generally the data have shown comparable survival, with reduced TRM but increased relapse in the recipients of the lower intensity preparative regimen [28,29]. In pediatric transplantation TRM is commonly lower than in adult studies, and the opportunity for improvement is therefore less. Data from National Marrow Donor Program show that TRM for children receiving unrelated donor transplant for acute leukemia has been cut in half in the last 10 years to 15%, likely as a consequence of improved HLA typing, larger donor pools for selection and improved supportive care [30,31]. In contrast, relapse rates have increased, suggesting that children with more aggressive leukemia are coming to transplant, and that a focus on improved disease control is more likely to improve outcomes than attempts to reduce TRM. Similarly, TRM for matched sibling donor bone marrow transplantation in CR1 for children transplanted in CR1 between 2006 and 2010 (n = 130) is 3% at day 100 and 6% at 1 year (personal communication from Mary Eapen, Center for International Blood and Marrow Transplant Research), so substituting reduced intensity for myeloablative transplant in the "upfront" setting could have only a modest effect on overall outcome.

Despite the low TRM seen in children reaching transplant, chemotherapy for AML is typically aggressive, and significant infections and impairments in organ function can arise and make fully ablative transplant risky. Reduced-intensity transplant may allow at least some of those children to be transplanted. It is important, however, to consider the data from such transplants critically. Getting a child to transplant at all costs only makes sense if we are sure that outcomes will be improved, and these data are currently lacking for children with biologically aggressive disease. Indeed, data from a combined analysis of transplantation of children with AML registered on a series of cooperative group studies did not show a survival advantage for children who managed to get to transplant, although this study included a relatively small number of transplants performed over a period of many years (26) [32].

APPROACHES TO IMPROVING OUTCOMES: GETTING CHILDREN TO TRANSPLANT: HYPOMETHYLATING AGENTS, AND NOVEL SMALL MOLECULES

Many children with high-risk AML will fail early in the course of treatment, dying early of toxicity (generally infection) or of refractory disease. Similarly, transplant studies of children with relapsed disease show 50% survival for those in second remission but fail to take into account children who never achieved a second remission, died of toxicity before remission, or entered remission with such severe organ dysfunction or infection they were not candidates for transplant. Newer chemotherapy agents such as decitabine or azacytidine offer alternative pathways to disease control with reduced toxicity and can facilitate transplant with a good performance status. Prospective studies are open in Cincinnati and in adult centers, offering a newer strategy that may be particularly applicable to biologically high-risk disease. Similarly, tyrosine kinase inhibitors and other small molecules are being tested, for example, Sorafenib for AML with FlT3-ITD. Incorporation of novel agents into pre- or post-transplant treatments may improve survival, and offers an attractive alternative to the successful but perhaps exhausted strategy of dose intensification using well-understood cytotoxic chemotherapy agents.

PREVENTION AND MANAGEMENT OF POSTTRANSPLANT AML RELAPSE

Relapse is the major cause of treatment failure after hematopoietic cell transplantation (HCT) for AML.

The majority of patients with morphologic relapse (>5% blasts) eventually succumb to their disease because posttransplant relapse therapies have been largely inadequate. An ongoing focus is to define earlier relapse thresholds based on sensitive techniques. The hypothesis is that interventions to treat MRD before morphologic relapse might mitigate the poor prognosis conferred by "MRD positive" (MRD+). Among several MRD monitoring parameters, MDF is the most broadly applicable technology that is available. MRD+ can be further delineated when other data are available. For example, disease-specific molecular markers, or serial chimerism studies that show declining donor hematopoeisis, particularly if lineage specific cell populations (eg, $CD34^+$) are analyzed [33,34]. Unfortunately, well-designed published intervention studies addressing AML relapse or MRD+ states before and after HCT, are lacking. We will focus on frequently asked questions about posttransplant relapse, whether MRD or morphologic relapse. A major caveat is that published intervention studies are retrospective, they suffer from selection bias, including time from HCT, tempo of relapse, sites of relapse, performance status, to list just a few.

How to React to MRD+ AML Immediately before HCT?

The decision to proceed in AML toward HCT relies heavily on the prognostic combination of well-described cytogenetic and molecular risk factors, patient performance status, and donor availability. Recently added to decision making for patients in morphologic CR is their end of induction and pretransplant MRD status. Walter et al. [35] recently showed that based on pretransplant MDF data, the 2-year survival for those with MRD immediately before HCT was 30% compared with that of 77% for MRD− patients. Similar findings were observed in children (15% versus 67%) [36]. One obvious approach is to try to reverse MRD+ status with additional chemotherapy. Unfortunately, the desired goal of MRD− may be met with further rise in MRD. Studies are needed to test whether the poor prognosis conferred by MRD+ AML is reversible even when MRD− is achieved. A valid concern is that cumulative toxicities and infections during chemotherapy attempts may increase transplant-related mortality. In this regard, it is worth remembering that among a large cohort of AML patients who were either in morphologic relapse or with primary induction failure, a 0 to ≥3 point score based on 5 adverse pre-HCT variables (first CR <6 months, circulating blasts, donor other than HLA-sibling or well-matched unrelated, Karnofsky Performance Scale/Lansky play performance status (LPP)S <90, and poor-risk cytogenetics) was able to delineate risk groups with 3- year survival ranging from 6% to as high as 42% [37]. Therefore, although pretransplant MRD+ associates with inferior survival, not getting to HCT as a result of failed attempts to convert MRD+ to MRD− likely reduces survival potential to 0%!

Can Conditioning be Augmented to Minimize Relapse?

One obvious strategy to combat posttransplant relapse is to augment conditioning in those at high risk of posttransplant relapse. Relapse rates decline when TBI doses are escalated but associated increases in nonrelapse mortality limit survival [27]. However, radiolabeled monoclonal antibodies that target radiation to hematopoietic tissues have been combined safely with RIC and show promising results [38]. The augmentation of a standard fludarabine (FLU)/Bu/antithymocyte globulin RIC with just 4 Gy TBI was shown also to lower relapse rates [39]. Recently, other groups also seeking to enhance the antileukemia effect of RIC have explored whether replacing FLU with clofarabine is beneficial and early data indicates excellent engraftment rates, reasonable safety, and promising efficacy in advanced AML [40,41].

What about Posttransplant Relapse Chemoprophylaxis?

Tyrosine kinase inhibitors have been used in BCR/ABL+ leukemias after HCT. Unfortunately, there is a paucity of available agents that target AML. Such agents should have drug interaction and toxicity profiles that are conducive to being used early after HCT. An unresolved but testable question is whether this approach should be used as prophylaxis in high risk, or preemptively in all, based on the earliest detection of MRD. Hypomethylating agents have been considered like low dose 5-azacitidine [42], which also has activity in childhood AML [43]. The FLT3 inhibitor sorafenib has been used in a small number of adults and children to prevent or treat MRD or frank relapse of FLT3 ITD mutated AML after HCT with a few notable CRs [44] (and unpublished observations). Trials to address safety, dose and length of therapy for these agents will be needed.

Should Immunosuppressive Therapy (IST) be stopped for Relapse?

Abrupt cessation of cyclosporine for early morphologic relapse after T cell-replete HCT has limited efficacy for diseases other than early phase chronic myeloid leukemia (CML) and most patients developed acute GVHD (aGVHD) within 2 weeks [45]. However, it is generally feasible to stop IST as soon as chemotherapy is begun. If GVHD emerges later then IST add back may be necessary. In response to 1 or more of withdrawal of IST, chemotherapy or donor leukocyte infusion (DLI), the Seattle group reported a CR rate of 30% among 307 patients in morphologic relapse,

of whom 80% had AML [46]. Median survival prolongation was 9.5 months if CR was achieved and 2-year overall survival based on time to relapse was 3% (<100 days), 9% (100-200 days), and 19% (>200 days). These data call into question the utility of curative intervention attempts, when morphologic (but not MRD+) relapses occur before 6 months and certainly before 3 months; thus, broaching the difficult discussion of palliative care is appropriate.

Should DLI be Used?

Unfortunately, the good results of DLI for relapsed CML do not generalize to other leukemias; survival at 2 years after DLI has been dismal to intermediate for AML. Factors in larger studies that were associated with the best survival (~50%-60%) were DLI in morphologic CR and/or favorable cytogenetics. Female patients or those not in CR but <35% blasts had survival of ~20%, and all others, 9% [47]. It is unclear how these risk factors hold up in the era of an expanding list of prognostic molecular markers (eg, FLT3, NPM1, CEBPa).

Although timing and cell dose considerations for DLI in CML have been well established, the more rapid tempo of AML relapse means that low-dose escalating DLIs are usually unfeasible. Reinduction to achieve CR is advised and the temporary lymphopenic state that results may facilitate homeostatic expansion of DLI. Although granulocyte colony stimulating factor primed DLI does not prevent aplasia (~20% of patients), some data suggest that granulocyte colony stimulating factor priming lowers aGVHD rates, with short courses of cyclosporine or methotrexate further adding to this effect without lessening chronic GVHD or increasing relapse rates [48]. Others have used granuloctye macrophage colony stimulating unit and/or low-dose interferon to try to augment the immunological graft-versus-leukemia (GVL) effect.

Whether prophylactic or preemptive DLI for high-risk AML is beneficial might be the subject of future cooperative group trials. An EBMT pediatric group used sensitive weekly monitoring for increasing mixed chimerism (recurrent host hematopoiesis) and intervened with discontinuation of IST and continued monitoring until complete chimerism was restored. For patients not on IST, or when mixed chimerism progressed after stopping IST, low-dose DLI was initiated. Although nonrandomized, the analysis showed 36% versus 0% survival, respectively, for those who did or did not receive DLI, which perhaps provides some proof of principle [49].

What about Extramedullary Relapse?

Extramedullary AML relapse appears more likely after prior aGVHD or chronic GVHD compared with those with marrow relapse, suggesting that GVL effects are more likely in the marrow. Patients with extramedullary relapse have better response to combined local and systemic therapy and improved survival compared with those with marrow relapse [50]. Therefore, local therapy should not be overlooked when extramedullary disease is present during plans for reinduction chemotherapy, DLI, and/or second transplant [50,51].

When Should Second Transplant be Considered?

Most published data on second HCT is becoming outdated but basic observations are worth reviewing. The multivariate analysis from an EBMT study of second allo-HCT (N = 177, 50% with AML) showed better 5-year survival for: relapse >292 days after first HCT, CR at second HCT, TBI with second HCT, and aGVHD after first and second HCT [52]. Eapen et al. [53] reported overall TRM of 42% and LFS 28% at 5 years; multivariate analyses showed improved outcomes for children (<20 years) and relapses >6 months from first HCT [53]. High-dose TBI/Cy can be safely given before second HCT in pediatric AML at least 6 months after high-dose Bu/Cy [54]. Although generally less relevant now, allogeneic after initial autologous HCT can result in 46% 2-year disease-free survival for AML, and favorable risk factors were: age <17 years, being in CR, and receiving TBI for the second HCT [55]. These data are helpful to consider because relapse is generally higher after RIC. However, children who have received high-dose TBI will need RIC options that currently include FLU and Bu, FLU/melphalan, or FLU/treosulfan. Augmentation of RIC platforms while preserving acceptable toxicity might involve replacing FLU with clofarabine, or adding radiolabeled monoclonal antibodies for better marrow targeting.

The notion that a different donor for second HCT may provide greater GVL has not been confirmed with the caveat than most studies have been underpowered to address this question.

SUMMARY AND FUTURE DIRECTIONS

There are no standard approaches and only limited information on therapeutic interventions other than DLI to treat relapse after HCT. Studies are needed to further characterize the significance of MRD, and particularly whether intervention can reverse the prognosis of pretransplant MRD+ AML. Relapse prophylaxis strategies are being explored in the form of targeted conditioning, minimally toxic posttransplant chemoprophylaxis, second transplants, and other approaches not discussed here due to time constraints. The latter include natural killer cell infusions, and naive T cell-depleted first HCT to try and abrogate aGVHD, thereby providing a feasible platform for natural killer, natural killer T, and central memory

T cells with potential to induce GVL hopefully unencumbered by high-dose steroids. One current drawback is the lack of large numbers of defined target antigens that can be exploited [56,57].

AUTHOR CONFLICT OF INTEREST STATEMENT

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